EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

Standard Operating Procedure
for
Media and Reagents Used in Efficacy Testing of Disinfectants

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1.0 SCOPE AND APPLICATION:

1.1 This SOP describes the procedures used to log-in, prepare, and evaluate the quality of media and reagents used in the disinfectant testing program.

2.0 DEFINITIONS:

2.1 MSDS = Material Safety Data Sheets

2.2 TSA = Tryptic Soy Agar

2.3 TGYE = Tryptone-glucose-yeast extract agar

2.4 M7H9 = Middlebrook 7H9 (agar or broth)

2.5 MPB = Modified Proskauer Beck

2.6 OSL = Organic Soil Load

3.0 HEALTH AND SAFETY:

3.1 All laboratory procedures are required to be performed in accordance with the biosafety practices stipulated in SOP MB-01, Biosafety in the Laboratory.

3.2 Since media preparation may require the use of heat producing lab equipment such as hot plates, steam sterilizers, and dishwashers, it is necessary to exercise extreme caution around these devices and any associated plumbing.

3.3 Technicians are required to wear a lab coat and protective eye-wear during the preparation and dispensing of media and reagents. Laboratory personnel must be trained on the proper use of autoclaves and dishwashers.

3.4 Several laboratory tasks discussed in this SOP involve the use of acids and bases; to protect against chemical burns, laboratory personnel must wear a lab coat, protective eye wear, and gloves during their use. Acid, bases and volatile chemicals will be handled according to the manufacturer's

instructions or information stated in the MSDS.

4.0 CAUTIONS:

4.1 Follow procedures outlined in SOP QC-03, Detergent Residues Test, for glassware washing procedures. If a detergent residues test identifies the presence of inhibitory detergent residues, then the wash procedure will be adjusted and the detergent residues test repeated until the wash procedure has proven that all inhibitory residues have been removed.

4.2 If the quality of the water used in the preparation of media and reagents falls outside of the acceptable limits as indicated in SOP QC-01, Quality of Purified Water, take corrective actions immediately as indicated in the SOP.

4.3 If the storage conditions for the media have been compromised in any way, then the media will be discarded. In addition, any media/reagent failing (unsatisfactory) a growth assessment or sterility verification will be discarded as indicated in SOP QC-11, Performance and Sterility of Media and Reagents.

4.4 Cultures of *Pseudomonas aeruginosa*, *Staphylococcus aureus, Mycobacterium bovis* (BCG) and *Bacillus subtilis* used in the performance evaluation of media must meet all required quality control elements established for each microbe as described in SOP MB-02, Test Microbes: Initiation, Maintenance and Quality Control.

4.5 The use of volumetric glassware is critical in the preparation of several media and reagents. Follow specific guidance described in the Media Recipe Book (see 16.1) and associated preparation sheets for details of situations where volumetric glassware must be used.

4.6 The pH of a specific medium or reagent must be adjusted to that specified by the manufacturer or the AOAC Official Methods of Analysis (see ref. 15.2).

5.0 INTERFERENCES:

5.1 Labeling of media and reagents must be accurate and legible. This includes control numbers, preparation control numbers, and sterilization batch numbers. If a preparation control number or sterilization batch number is illegible or missing and cannot be determined from the Media/Reagent Preparation Sheet (see 16.4) or the Daily Sterilization Record Information Log Form (see 16.5), the media or reagent will not be used in any testing procedure and will be discarded. Thus, illegible entries may cause interference with media and reagent quality control.

5.2 All pre-sterilized laboratory supplies will be inspected upon receipt for damage or torn packaging. Cracked petri-dishes or pipettes may puncture packaging and allow contaminating organisms to enter. Furthermore, pre-sterilized laboratory supplies will be assayed for sterility prior to use according to procedures described in SOP QC-12, Sterility of Pre-Sterilized and Autoclaved Supplies. Undetected contamination of pre-sterilized supplies may interfere with the interpretations of media performance and sterility tests.

5.3 Detergents used in washing glassware that is used in the preparation of media and reagents may leave residues which are bacteriostatic. Undetected residues on glassware may interfere with the interpretations of media and reagent performance tests. Nonconformance standards have been established and will be followed. Refer to SOP QC-03, Detergent Residues Test, for procedures regarding glass washing and the detergent residues test.

6.0 PERSONNEL QUALIFICATIONS:

6.1 Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

7.0 SPECIAL APPARATUS AND MATERIALS:

7.1 Equipment and materials needed to prepare and evaluate the sterility and performance of media and reagents are specified in the SOPs referenced in this document.

8.0 INSTRUMENT OR METHOD CALIBRATION:

8.1 Equipment used in the preparation of media and reagents are subject to calibration and maintenance as described in the following SOPs: EQ-01, pH Meters; EQ-03, Weigh Balances; EQ-06, Burets; EQ-08, Media Dispensors; and QC-13, Performance Verification of Autoclaves. See section 10.3 of this SOP for more details.

9.0 SAMPLE HANDLING AND STORAGE:

9.1 Media and reagents are subject to proper storage as specified by the manufacturer. Refer to SOP QC-10, Expiration Time and Examination of Media and Reagents, for details on the storage and expiration dates for prepared media and reagents.

10.0 PROCEDURE AND ANALYSIS:

10.1 Receipt, Identification, and Labeling of Media and Reagents.

10.1.1 All chemicals, media, prepared or purchased reagents, and pre-sterilized laboratory supplies will be logged-in upon receipt and assigned a control number. The products are marked with a label containing the control number and the date the product was opened. This information should be clearly legible on the labeled container. Refer to SOP QC-09, Control Numbers, for details of the log-in process.

10.2 Preparation of Media and Reagents Used in the Testing Program.

10.2.1 The specific directions for preparation of media and reagents are contained in the Media Recipe Book (see 16.1). The directions will be strictly followed. When preparing media and reagents, completion of the Media/Reagent Preparation Sheet is required (see 16.4). Partially completed Media/ Reagent Preparation Sheets may also be used (see 16.2). In addition, several media require the use of a water bath to hold media at a prescribed temperature; refer to SOP QC-14, Monitoring Temperature of Water Baths, for directions.

10.2.2 The following sections identify specific media and reagents used in the laboratory, the associated SOP and/or the Media

Recipe Book (see 16.1) for reference purposes.

10.2.2.1 Synthetic Hard Water: If a product makes hard water claims, then AOAC Synthetic Hard Water is prepared as described in the Media Recipe Book.

a. Hard water reagents: Solutions 1 & 2 and Solutions A through E

10.2.2.2 Organic Soil Load (OSL): If a product makes a claim for an OSL, then the OSL is prepared as described in the Media Recipe Book.

a. Horse serum
b. Fetal bovine serum
c. Bovine albumin fraction V solution

10.2.2.3 Neutralizers: Test parameters will include the use of a specific neutralizer, the neutralizer is prepared as described in the Media Recipe Book.

a. Letheen broth
b. Horse serum
c. AOAC neutralizer stock solution
d. AOAC neutralizer blanks
e. FTM

10.2.2.4 SOP QC-02, Air/Surface Monitoring.

a. TSA

10.2.2.5 SOP QC-03, Detergent Residues Test.

a. TGYE
b. Nutrient broth
c. Synthetic broth
d. Phosphate buffered dilution water

10.2.2.6 SOP QC-04, Cleaning Recirculating Chillers.

a. TGYE
b. TSA

10.2.2.7 SOP QC-10, Expiration Time and Examination of Media and Reagents.

a. All media and reagents: see Media Recipe Book

10.2.2.8 SOP QC-11, Performance and Sterility of Media and Reagents.

a. All media and reagents; see Media Recipe Book

10.2.2.9 SOP QC-12, Sterility of Pre-Sterilized and Autoclaved Supplies.

a. Fluid thioglycollate medium

10.2.2.10 SOP QC-18, Sterility of Disinfectants.

a. Fluid thioglycollate medium
b. Letheen broth
c. TSA

10.2.2.11 SOP MB-02, Test Microbes: Initiation, Maintenance and Quality Control.

a. Cystine trypticase agar
b. Mannitol salt agar
c. MacConkey's agar
d. Nutrient broth
e. Nutrient agar
f. Synthetic broth
g. Pseudosel agar
h. TSA
i. M7H9 agar
j. MPB

10.2.2.12 SOP MB-03, Screening Carriers.

a. BTC 835
b. Asparagine solution
c. 1 N NaOH

10.2.2.13 SOP MB-04, Carrier Counts.

a. Letheen broth
b. M7H9 agar
c. TSA
d. MPB
e. Phosphate buffered dilution water

10.2.2.14 SOP MB-05, Use Dilution Method.

a. Asparagine solution
b. Nutrient broth
c. Synthetic broth
d. Disinfectant sample preparation
e. MacConkey's agar
f. Mannitol salt agar
g. Pseudosel agar
h. TSA

10.2.2.15 SOP MB-06, Testing Spray Disinfectants.

a. Nutrient broth
b. Synthetic broth
c. TSA
d. MPB
e. 0.1% Tween 80 in saline
f. Letheen broth
g. Disinfectant sample preparation
h. M7H9 broth
i. M7H9 agar
j. Kirchners medium
k. TB broth
l. MacConkey's agar
m. Mannitol salt agar
n. Pseudosel agar

10.2.2.16 SOP MB-07, Confirmatory Tuberculocidal Method.

a. 0.1% Tween 80 in saline
b. MPB
c. M7H9 agar
d. M7H9 broth
e. Kirchner's medium
f. TB broth

10.2.2.17 SOP MB-09, Testing Towelette Disinfectants.

a. Nutrient broth
b. Synthetic broth
b. TSA
c. MPB
d. 0.1% Tween 80 in saline
e. Letheen broth
f. Disinfectant sample preparation
g. M7H9 broth
h. M7H9 agar
i. Kirchner's medium
j. TB broth
k. MacConkey's agar
l. Mannitol salt agar
m. Pseudosel agar

10.2.2.18 SOP, FDA Protocol for Testing Sporicidal Activity.

a. Fluid thioglycollate medium
b. Modified fluid thioglycollate medium
c. Letheen broth
d. Nutrient broth
e. Nutrient agar slants
f. Sodium hydroxide
g. Hydrochloric acid
h. TSA
i. Phosphate buffered dilution water

10.2.2.19 SOP MB-12, Sporicidal Neutralization Test.

a. Fluid thioglycollate medium
b. TSA
c. Phosphate buffered dilution water
d. Sterile de-ionized water

10.3 Quality control activities associated with the preparation of media and reagents.

10.3.1 Equipment used in the preparation of media and reagents is subject to calibration and maintenance as described in the following SOPs:

SOP EQ-01, pH Meters
SOP EQ-03, Weigh Balances
SOP EQ-06, Burets
SOP EQ-08, Media Dispensors
SOP QC-13, Performance of Autoclaves

10.3.2 The quality of de-ionized water used in the preparation of media and reagents is verified according to the methods described in SOP QC-01, Quality of Purified Water.

10.3.3 A Detergent Residues Test will be performed annually on each dishwasher or when a new or differing lot of detergent is used. This test determines the presence of bacteriostatic detergent residues which may remain following washing of glassware. Refer to SOP QC-03, Detergent Residues Test, for procedures regarding glass washing and the detergent residues test.

10.4 Labeling.

10.4.1 Tracking media and reagents from preparation to use must be thoroughly documented. Each preparation of media or reagent will be clearly labeled with the name of the preparation and the preparation control number. When preparing media and reagents, completion of the Media/

Reagent Preparation Sheet is required (see 16.2, 16.4). Refer to SOP QC-15, Media Prep and Sterilization Run Numbers, for details for assigning media preparation control numbers and sterilization batch control numbers (see 16.5).

10.5 Performance and Sterility.

10.5.1 Testing must be performed to assess the ability of the media to support recovery/growth of the test organisms and to verify media/reagent sterility. Test methods are described in SOP QC-11, Performance and Sterility of Media and Reagents.

10.6 Shelf-life.

10.6.1 SOP QC-10, Expiration Time and Examination of Media and Reagents, establishes expiration dates for media and reagents used in conjunction with efficacy testing of disinfectants, and describes the procedure for visual assessment of stored media.

11.0 DATA ANALYSIS/CALCULATIONS:

11.1 Refer to Section 11.0, DATA ANALYSIS/CALCULATIONS, of the SOPs referenced in this document for specific details.

11.2 If other than the standard amounts of ingredients specified in the recipe are used to prepare media and reagents, the calculations should be shown on the prep sheets. For example, if two liters of fluid thioglycollate medium are made, the prep sheet should show the calculation of 29.8g for 1 liter multiplied by 2: 29.8 x 2 = 59.6g dehydrated medium required to prepare 2 liters of fluid thioglycollate medium.

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 Data will be recorded promptly, legibly, and in indelible ink on all forms associated with the SOPs referenced in this document. Completed forms

are archived in notebooks kept in locked file cabinets in file room D217. Only authorized personnel have access to the locked files. Archived data is subject to OPP's official retention schedule contained in SOP ADM-03, Records and Archives.

13.0 QUALITY CONTROL:

13.1 The OPP Microbiology Laboratory conforms to 40CFR Part 160, Good Laboratory Practices (see ref. 15.1). Appropriate quality control measures are integrated into each SOP.

13.2 For quality control purposes, the required information is documented on the appropriate forms.

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 Refer to Section 14.0, NONCONFORMANCE AND CORRECTIVE ACTION, of the SOPs referenced in this document for specific details.

15.0 REFERENCES:

15.1 EPA Good Laboratory Practice Standards, 40 CFR Part 160.

15.2 Official Methods of Analysis. 1990. 15th Ed., Association of Official Analytical Chemists, Arlington, VA, Method 955.15, 961.02, 964.02, 965.12, and 966.04.

15.3 FDA Protocol for Testing Sporicidal Activity. June, 1993.

16.0 FORMS AND DATA SHEETS:

16.1 Attachment A: Media Recipe Book

16.2 Attachment B: Partially Completed Media/Reagent Preparation Sheets

16.3 Attachment C: FDA SOP for Sporicidal Activity Test

16.4 Blank Media/Reagent Preparation Sheet

16.5 Daily Sterilization Record Information Log Form

Attachment A: Media Recipe Book

Media Recipe Book on file in the OPP Microbiology Laboratory file room (D217).

Attachment B: Partially Completed Media/Reagent Preparation Sheets

Partially Completed Media/Reagent Preparation Sheets on file in the OPP Microbiology Laboratory file room (D217).

Attachment C: FDA SOP for Sporicidal Activity Test

FDA SOP for Sporicidal Activity Test on file in the OPP Microbiology Laboratory file room (D217).

Media/Reagent Preparation Sheet
OPP Microbiology Laboratory

Media/Reagent Name:	
Amount Prepared:	Preparation Date/Initials:
Prep #:	Sterilization Number:

Media/Chemical Ingredients:	Control No:	Amount Required:	Amount Weighed:

Preparation/Modifications/Notes:

Required pH:	Original pH:	Final pH: Temperature:

Volume of Acid/Base added to obtain final pH:	

Sterility/Viability Test Results	Sterility		Viability	
	Pass	Fail	Pass	Fail

Storage of Reagent/Media:

Daily Sterilization Record Information Log Form

OPP Microbiology Laboratory

Cycle[1]		Unit Temp.[2]		Max. Thermometer[3]		Chemical Indicator Strips			Items Processed	Sterilization No.[5]	Init.
Type	Time	Min.	Max.	Temp.	Serial #	Lot No.	Exp. Date	Result[4]			

1 Record the cycle as “G” = gravity, “L” = liquid under Type and the duration of the cycle in minutes under Time.
2 Record the maximum and minimum temperature achieved during the sterilize phase of the cycle as indicated by the autoclave printout (Unit).
3 Record the corrected value for the maximum registering thermometer (Max.) and the serial number of the thermometer.
4 Record the results of the chemical indicator strips as “P” for pass or “F” for fail.
5 The Sterilization No. indicates the date as well as the unit location and the run number.